REMARKS

Reexamination and further and favorable reconsideration of the subject application in light of the following remarks, pursuant to and consistent with 37 C.F.R. § 1.112, are respectfully requested.

Claims 1-10, 12, 22, 26, 40, 42-44, 46, 50, 53, 54, 56, 58, and 63-108 are pending. Claims 1-10, 12, 22, 26, 40, 43, 44, 46, 50, 70-84, 88-99, 104 and 105 have been withdrawn from consideration. Claims 22, 26, 42, 53, 54, 56, 58, 63-69, 85-87, 100-103 and 106-108 have been rejected on new grounds. As no other grounds for rejection have been addressed in the present Office Action, it is understood that all other previously alleged grounds for rejection have been withdrawn.

New Rejection under 35 U.S.C. § 103

Claims 22, 26, 42 53, 54, 56, 58, 63-69, 85-87, 100-103 and 106-108 have been rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Fire et al. (USPN 6,506,559) in view of Brown et al. (US patent 5,859,347) and Lusky et al. (US patent 6,350,575), the combination in view of Barracchini et al. (US Patent 5,801,154).

The rejection is respectfully traversed, because the cited references do not support a prima facie case of obviousness. The references cited by the Office in support of the rejection, fail to teach or suggest all the elements of the presently claimed invention, and fail to provide a motivation to a person of ordinary skill in the art to combine and modify the cited references as proposed by the Office. Moreover, the references, taken together, fail to recognize the surprising results that may be obtained by the present invention.

The Office alleges that Fire et al. teaches plant cells, plants and their seeds comprising a nucleic acid comprising a first and second DNA sequence which expresses in the plant cell

a chimeric DNA comprising a promoter, operatively linked to a DNA region which, when transcribed, yields an RNA molecule capable of forming a hairpin comprising two annealing RNA sequences which comprise a sense sequence sharing homology with consecutive nucleotides of a target nucleic acid of interest in the plant, and which further comprises a second, annealing RNA sequence comprising antisense sharing homology with the consecutive nucleotides of the sense strand that targets the nucleic acid of interest, and which chimeric DNA further comprises an intron sequence, and which chimeric DNA further comprises operably linked transcription termination and polyadenylation sequences.

Among the distinctions between Fire et al. and the presently claimed inventions, the Office has acknowledged that:

a) Fire et al. do not teach the intron to be interposed between the sense and antisense strand of the chimeric construct; and

b) Fire et al. do not teach the targeting region of the chimeric construct to span between 10 and 50 consecutive nucleobases and to share between 75 to 100% identity between the sense strand and the target sequence.

However, the Office ascribes more to Fire et al. than is actually taught by the reference. Fire et al. teach the introduction of dsRNA into *Caenorhabditis* to achieve gene silencing of target genes. At best, Fire et al. suggest that the dsRNA sequences could be present in an RNA molecule that could be expressed from a single gene. However, contrary to the allegation of the Office, Fire et al. are completely silent about the possibility of such a gene containing an intron sequence. Even the passages of Fire et al. cited by the Office on page 4 of the current Official Action that do mention the word "intron" do so only in a completely different context. For example, at column 5, lines 40-4, Fire et al. merely indicate that targeted genes can contain exons and introns, and at column 17, lines 20-25, Fire et al.

teach that dsRNA segments matching intron sequences in target genes did not produce detectable inhibition. Thus, one would be lead away from including intron sequences in a dsRNA. In any case, this disclosure certainly does not amount to a teaching or suggestion to include intron sequences in chimeric genes encoding dsRNA.

Thus, Fire's disclosure not only differs from the current claims in the cited range of nucleotides and sequence identity, and in the position of the intron sequence, Fire et al. does not even suggest including **an intron as such** in a gene encoding dsRNA (to the extent they even suggest a dsRNA encoding gene).

The combination of secondary references cited by the Office in support of the rejection fails to remedy the deficiencies of Fire et al.. Indeed, Fire et al. has been previously considered by the Office in the context of the related prior art. *See*, Office Action mailed April 9, 2003 at page 7.

> "US Patent No 6506559 (Fire et al) is considered to be pertinent to Applicant's disclosure because it discloses methods of inhibiting the expression of a target gene using a dsRNA molecule, including an RNA molecule expressed from a DNA vector, **however, Fire et al. does not teach these methods wherein the DNA vector comprises a heterologous intron, nor is there any suggestion in the prior art to include such an intron in the DNA vectors expressing a dsRNA of Fire et al."** *Id.* (emphasis added).

Thus, the Office has already considered Fire et al. in view of the state of the art at the time the application was filed and found that the prior art does not remedy the deficiencies inherent in Fire et al.; and thus, the Office correctly found that the instant claims are patentable over the prior art.

Among the secondary references now cited by the Office, Brown et al. is cited for its alleged teaching of plant cells transformed with chimeric nucleic acid expression constructs expressing desired DNA sequences, which expression constructs include operably linked promoters and further comprising heterologous introns, which introns enhance expression of

the desired nucleic acid sequences in the expression construct. However, Brown et al. does not teach or suggest a construct expressing a dsRNA and comprising a heterologous intron arranged as in the presently claimed invention. Accordingly, Brown et al. fail to provide any motivation to modify Fire et al. as proposed.

Brown et al. is directed at inclusion of particular introns sequences into the **non-translated leader** of a chimeric gene with the aim to express greater **quantities of proteins in plants**. See, Brown et al. at column 1, lines 10-12. Brown et al. teaches the importance of locating the intron in the untranslated leader portion of the chimeric gene, thus directing a person of ordinary skill in the art away from even trying including an intron in a chimeric gene arranged as in the presently claimed invention.

Further, there is no indication in Brown et al. that the observed increased expression was due to increased transcription, or an increased level of the mRNA. As the Examiner will realize, the biologically active molecule for the reduction of the expression of the target gene in the current application is the transcribed RNA which can produce a double stranded RNA molecule, no protein is expressed from the claimed chimeric gene. Thus, the increase in protein expression observed by Brown et al. would have no relevance to the presently claimed invention and would provide no motivation to combine the references as proposed by the Office.

There is also no indication in Fire et al. that increased expression of a chimeric gene encoding a dsRNA molecule would lead to increased silencing of the target gene. To the contrary, what Fire et al. do indicate is that low concentrations of RNA molecules are sufficient for the purposes of dsRNA mediated gene silencing (column 5, lines 15 to 17). In other words, there is no incentive provided by the Fire et al. disclosure to increase the expression of a gene encoding a dsRNA molecule to improve the silencing of the expression

of the target gene, even if the teachings of Brown et al. could be read to suggest that an intron might increase production of RNA, which they cannot.

Lusky et al. is also cited for its alleged teaching of expression constructs comprising antisense RNA and further teaching that expression construct can comprise an intron as well as other expression elements including translation termination and polyadenylation signals. What Lusky et al. provides is teaching concerning the construction of viral expression vectors and helper cells. Genes in these constructs can contain intron sequences. However, Lusky et al. do not teach or suggest the arrangement of elements in the presently claimed invention and fail to provide any motivation to modify Fire et al. as proposed. Even if vectors exist in the prior art containing heterologous DNA with intron sequences, they have no clear relevance to improving dsRNA silencing, and certainly do not provide any motivation to include an intron as arranged in the present claims.

Finally, Baracchini et al. is cited for its teaching of the ability to target a gene of interest with a complementary sequence comprising at least 10 nucleobases. However, Baracchini et al. fails to remedy the deficiencies of the combination of Fire et al. with Brown et al. and Lusky et al. In the absence of any teaching or motivation to modify Fire et al. as proposed, there would be no motivation of a person of ordinary skill in the art to consult a reference such as Baracchini et al.

Thus, in addition to the distinctions already recognized by the Office, Fire et al. do not teach the inclusion of any introns into the chimeric dsRNA encoding constructs designed to silence the phenotypic expression of a target gene. In fact, the only teaching by Fire et al. concerning introns is that the dsRNA targeted to intron sequences of the target genes are not effective in achieving inhibition of the target gene (see column 17, lines 20-22).

Brown et al. teach inclusion of an intron in the untranslated leader region to improve protein expression in plants. Brown et al. do not teach that increased protein expression is due to increased transcription or increased levels of RNA. There is no incentive provided by the Fire et al. disclosure to suggest increasing the expression of a chimeric gene encoding the dsRNA molecule, even if Brown et al. could be read as suggesting that an intron could do this, which Brown et al. does not even suggest.

Furthermore, Fire et al. in column 3, lines 19-34 states that "inhibition by dsRNA must occur by a mechanism distinct from antisense interference". Therefore, at the time the invention was made, the person skilled in the art would not have extrapolated any purported results of antisense constructs which the Office alleges may be ascribed to Lusky et al. to be to be relevant to dsRNA mediated gene silencing.

Baracchini et al. was cited only for allegedly teaching sizes and amounts of sequence identity between the dsRNA and the target gene to be silenced. Applicants respectfully submit that in the light of the above, with regard to the presence of an intron or heterologous intron into the dsRNA encoding chimeric gene, it will be clear that the currently claimed subject-matter is not obvious over the prior art irrespective of the claimed size ranges and sequence identities and no further discussion of Barrachini et al. is required.

For at least the foregoing reasons, one of ordinary skill in the art would not have been motivated to include intronic sequences within the dsRNA encoding chimeric genes by the teachings of Brown et al, Lusky and Fire et al.

Further, taking the cited references together, a person or ordinary skill would not have expected that inclusion of intronic sequences in a dsRNA encoding chimeric gene would result in a more efficient reduction of the phenotypic expression of a nucleic acid of interest. There is nothing in the cited references that suggests an appreciation of the results that can be

obtained by using the presently claimed invention or any proposed reason for the enhancement observed. Applicants would like to draw the attention of the Office to the attached published report by a group of authors including Applicants for the current application. Smith et al., Nature, 407:319-32, 2000 (Attached as Exhibit A). The report shows that presence of an intron enhances silencing efficiency by either

a. increased formation of duplex RNA during the process of intron excision from the construct by the spliceosome due to better alignement of the complementary arms of the hairpin in an environment favouring RNA hybridization;

b. increased retention of hairpin RNA in nucleus; or

c. creation of a smaller, less nuclease-sensitive loop.

Id. at 320, paragraph spanning the 1st and 2nd column).

None of these potential benefits attached to the inclusion of introns is suggested by Fire et al., Brown et al., and Lusky et al.

Thus, the art cited in support of the rejection fails to establish a prima facie case of obviousness, at least in failing to provide any motivation to combine and modify the cited art as proposed by the Office. Moreover, the art fails to indicate any appreciation of the results that can be obtained or any proposed basis of such results. For at least these reasons, withdrawal of the rejection is respectfully requested.

CONCLUSION

In view of the foregoing, further and favorable action in the form of a Notice of Allowance is believed to be next in order. Such action is earnestly solicited.

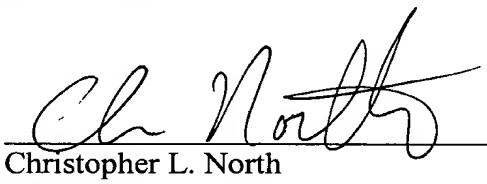
In the event that there are any questions relating to this application, it would be appreciated if the Examiner would telephone the undersigned concerning such questions so that prosecution of this application may be expedited.

The Director is hereby authorized to charge any appropriate fees that may be required by this paper, and to credit any overpayment, to Deposit Account No. 02-4800.

Respectfully submitted,

BUCHANAN INGERSOLL PC

Date: September 12, 2005

By: 

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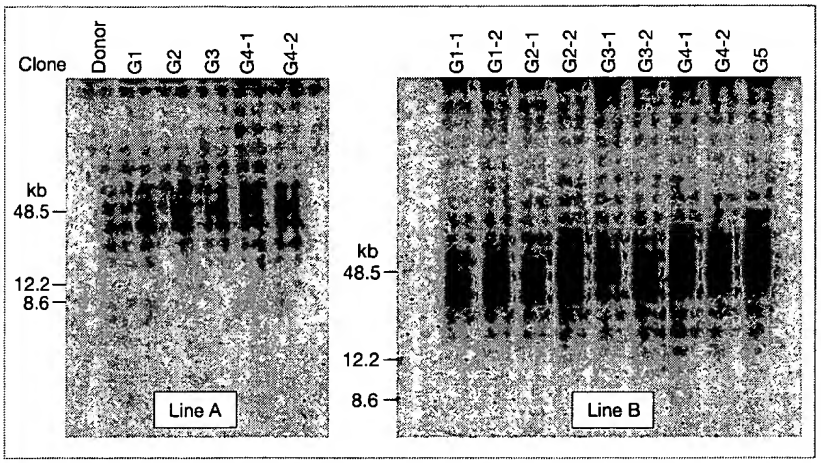

Figure 1 Telomere lengths in successive generations (G1–G5) of mice cloned from cumulus cells. Southern-blot analysis of terminal restriction-enzyme-cut fragments in five sequential generations shows that telomeres do not undergo incremental erosion in successive clonal generations. Genomic DNA isolated from peripheral-blood lymphocytes taken from representative animals from each generation was digested with the restriction enzyme *HinfI*, resolved on a pulse field gel, transferred to a solid support and probed with a 5′-^{32}P-labelled $(T_2AG_3)_3$ oligonucleotide. Peripheral blood lymphocytes were sampled on the same day. Ages of mice (in months) were: in line A, donor, 18; G1, 16; G2, 14; G3, 12; G4, 9; G5, 9; in line B, G1, 15.5; G2, 13; G3, 11; G4, 9; G5, 7. Suffix numbers (G4-1 and G4-2, for example) identify different pups of each generation.

was repeated with cumulus cells from adult G1 mice as nucleus donors to produce the next clonal generation, G2, and so on. Table 1 summarizes the results obtained following the reconstruction of 3,920 enucleated oocytes.

Previously, about 2% of enucleated oocytes receiving a cumulus cell nucleus developed to live-born pups[1]. This value is comparable to the cloning efficiency for G1 in lines A (1.5%) and B (4.2%). However, the success rate dropped in successive cloned generations: line A did not produce a G5 clone from 670 reconstructed oocytes; in line B, the only live-born G6 clone was cannibalized by her foster mother, thereby terminating the line. Mouse lines A and B therefore represent totals of 9 and 26 clones from their respective donors. Placental size was consistently in the range previously reported for cloned mice[2] and did not increase in successive generations (data not shown).

Do sequentially cloned mice show signs of accelerated ageing? We assessed the behaviour of these mice and determined telomere lengths to assess organismal and cellular measures of ageing, respectively. We evaluated learning ability in the Morris water maze and Krushinsky tests, as well as strength and agility, and also used other assays designed to monitor signs of premature ageing, such as a decline in activity in the home cage and loss of coordination[4]. All cloned mice were, by these criteria, normal compared with age-matched controls (data not shown); the G5 mouse is alive and healthy at 1.5 years.

We measured telomere length in peripheral blood lymphocytes of clones G1–G6 by Southern-blot analysis of terminal restriction-enzyme-digested fragments (Fig. 1) and found no evidence of shortened telomeres in the cloned mice. In fact, our results show that the telomeres lengthen with each generation. As representative animals of each generation were sampled simultaneously, we cannot rule out an age-related contribution to this increase (with younger mice having longer telomeres). In addition, long telomeres in mice are optimally studied by means of different assays such as quantitative fluorescence *in situ* hybridization[5]. We have detected telomerase activity in cumulus cells (data not shown); it is therefore possible that telomeres in these cells are unusually long, resulting in offspring with concomitantly longer telomeres.

Shortened[6] and lengthened[7] telomeres have been reported in cloned livestock but, unlike ours, those experiments involved only a single round of cloning. Our results on sequentially cloned mice verify that telomere shortening is not a necessary outcome of the cloning process[8]. However, as only 1–2% of reconstructed oocytes yield live-born clones, the possibility of selection for donor nuclei with the longest telomeres cannot be excluded. Further investigation is required into the consequences of nuclear transfer on telomere length and lifespan.

Table 1 Effect of sequential cloning on full-term development

Line	G1	G2	G3	G4	G5	G6	Total
A	2/131 (1.5)	1/228 (0.4)	1/263 (0.4)	5/238 (2.1)	0/670 (0)	-	9/1,530 (0.6)
B	4/96 (4.2)	7/351 (2.0)	5/352 (1.4)	6/286 (2.1)	3/581 (0.5)	1/724 (0.1)	26/2,390 (1.1)

Successive generations are represented as G1, G2 and so on for two independent mouse lines, A and B. The number of pups born live after cumulus-cell nuclear transfer is compared to successfully reconstructed oocytes (pups/oocytes), with the corresponding percentages in parentheses. Significant χ^2 comparisons were derived for G4 and G5 from line A, G1 and G5, G6 from line B, and G2, G3, G4 versus G6 from line B ($P < 0.05$).

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Gene expression

Total silencing by intron-spliced hairpin RNAs

Post-transcriptional gene silencing (PTGS), a sequence-specific RNA degradation mechanism inherent in many life-forms, can be induced in plants by transforming them with either antisense[1] or co-suppression[2] constructs, but typically this results in only a small proportion of silenced individuals. Here we show that gene constructs encoding intron-spliced RNA with a hairpin structure can induce PTGS with almost 100% efficiency when directed against viruses or endogenous

genes. These constructs could prove valuable in reverse genetics, genomics, engineering of metabolic pathways and protection against pathogens.

Induction of PTGS by co-suppression and antisense methods that target the Nia-protease (*Pro*) gene sequence of potato virus Y (PVY)[3] cause silencing in 7% and 4% of independent transformants, respectively; induction of PTGS in these tobacco plants (*Nicotiana tabacum*) manifests as immunity[3,4] to the virus.

Using principles we developed for silencing constructs that express double-stranded RNA and inverted-repeat RNAs[3], we made a construct encoding a single self-complementary hairpin RNA (hpRNA) of the *Pro* sequence. The construct contains sense and antisense *Pro* sequences flanking an 800-nucleotide spacer fragment derived from the *uidaA* (GUS) gene (Fig. 1a). About 60% (25/43) of the plants that are transformed with this construct, many of which contained a single transgene copy, were immune to the virus. The spacer fragment contributed to the stability of the perfect inverted-repeat sequences, but it was not required for the specificity of the PTGS (Fig. 1a).

To test the effect of removing the loop region of hpRNA, we replaced the spacer with an intron sequence (Fig. 1a, b). The intron sequence provides stability to the DNA, but is spliced out during pre-mRNA processing[5] to produce loopless hpRNA. As a control, we made a sister construct in which the intron sequence was inserted in the reverse, non-splicing, orientation. When transformed into tobacco, 22 of 34 (65%) reverse-intron plants were immune, a similar frequency to plants transformed with the GUS spacer construct. Amazingly, we found that 22 of 23 plants transformed with the construct containing the functional intron were immune to the virus.

To test whether this enhancement by the sense-intron construct was a general phenomenon, we made two hpRNA constructs against the endogenous Δ12-desaturase (*Fad2*) gene of *Arabidopsis*, which catalyses the conversion of oleic to linoleic acid in the seed[6,7]; one construct contained an intron and the other a non-intron spacer region. We found that 69% (44/63) of the transgenic plants with the non-intron spacer region construct showed PTGS of the Δ12-desaturase gene, but that 100% (30/30) of plants transformed with the intron construct showed silencing of this gene.

How does the presence of this intron enhance silencing efficiency? The process of intron excision from the construct by the spliceosome might help to align the complementary arms of the hairpin in an environment favouring RNA hybridization, promoting the formation of a duplex. Alternatively, splicing may transiently increase the amount of hairpin RNA by facilitating, or retarding, the hairpin's passage from the nucleus, or by creating a smaller, less nuclease-sensitive loop.

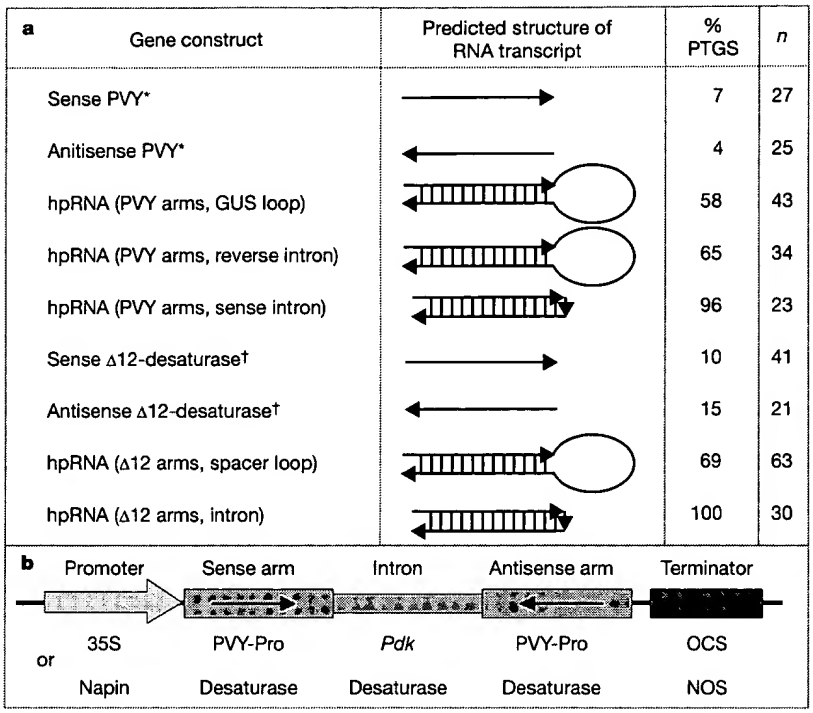

Figure 1 Efficiency of induction of post-transcriptional gene silencing (PTGS) by different gene constructs and the predicted structure of RNA transcribed from the transgenes. **a**, PTGS efficiency measured for potato virus (PVY) and Δ12-desaturase genes as the percentage of independent transgenic plants immune to PVY and the percentage of plants with enzyme activity reduced by more than 20% compared with wild type, respectively. In the predicted structures of RNA transcripts, right- and left-pointing arrows represent sense and antisense orientation of sequences, respectively; small vertical arrows represent splice-junction sequences remaining after the intron has been spliced out. Vertical lines in the predicted structures indicate duplex formation. Asterisks, data from ref. 3; daggers, data from ref 7; hpRNA, hairpin RNA; *n*, number of independent transformants; GUS, β-glucuronidase. **b**, Design of intron-containing hairpin constructs. OCS, octopine synthase; NOS, nopaline synthase.

We believe that constructs encoding intron-hpRNA should efficiently induce PTGS for a wide range of genes in a variety of circumstances and could become as useful to plant biology as RNAi[11,12] is to the study of nematodes and *Drosophila*. The transgene design might also have application in organisms in which RNAi has been obtained by injection of double-stranded RNA.

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